

**Amendments to the Specification:**

Please replace the paragraph beginning on page 3, line 14, which starts with "Fig. 3-a", with the following amended paragraph.

Fig. 3-a shows the elution pattern of plant growth factors in DEAE Sephadex A-25™ (a 2-(diethylamino)ethyl-moiety containing resin) ion-exchange chromatography; Fig. 3-b shows the elution pattern of plant growth factors in Bio-Gel P-2™ extra fine chromatography; and Fig. 3-c shows the elution pattern of plant growth factors in Develosil ODS-HG-5™ reversed-phase HPLC.

Please replace the paragraph beginning on page 5, line 20, which starts with "An anion-exchange resin", with the following amended paragraph.

An anion-exchange resin such as DEAE Sephadex A-25™, DEAE cellulose and DEAE Sepharose™ is swollen with a buffer (pH of 6.5 to 8.0) such as 10 to 100 mM tris-HCl buffer, phosphate buffer and sodium carbonate-carbonic acid buffer. Then, the plant growth factors contained in CM are adsorbed to the anion-exchange resin by a column method or a batch method. Next, the plant growth factors may be collected through elution with salts such as potassium chloride and sodium carbonate, with stepwise increasing the salt concentration from 10 to 2000 mM. The plant growth factors are eluted at a salt concentration of 800 to 2000 mM, preferably 1000 to 1250 mM.

Please replace the paragraph beginning on page 6, line 7, which starts with "The resulting fractions", with the following amended paragraph.

The resulting fractions are then desalted through dialysis or the like, and thereafter applied to a gel permeation column, preferably Bio-Gel P2™, Bio-Gel P4™ (both produced by Bio Rad), Sephadex G25™ (produced by Pharmacia) or the like.

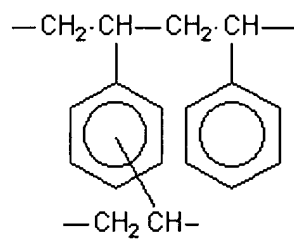
Please replace the paragraph beginning on page 6, line 10, which starts with "The active fractions", with the following amended paragraph.

The active fractions thus desalted through such dialysis and gel permeation are then fractionated using reversed-phase high performance liquid chromatography (reversed-phase HPLC) with Nucleosyl-100-C18™ (produced by Nagel), Microsolve PR18™ (produced by Merck) or the like as the carrier. The purified plant growth factors are obtained from the fractions at a retention time between 8 and 12 minutes, which are eluted with a solvent of water-acetonitrile, water-methanol, water-ethanol or the like containing trifluoroacetic acid (TFA).

Please replace the paragraph beginning on page 16, line 16, which starts with "Cosmosil", with the following amended paragraph.

Cosmosil 75C<sub>18</sub>-OPN™ (porous spherical silica particles with an average size of 75  $\mu$ m and an average pore size of 120 Å (10 g)(Nacalai Tesque) was suspended in methanol, degassed in a vacuum, and filled into a column (1.7 x 8 cm; 18 ml). After the carrier was filled completely, the eluent in the column was replaced with distilled water, and the column was washed with 100 ml of distilled water. Then, 5.0 ml of CM was put

into the column, and eluted with 100 ml of distilled water, 100 ml of 30% CH<sub>3</sub>CN, and 100 ml of 60% CH<sub>3</sub>CN in order (flow rate: 60 ml/hour). The resulting fractions were evaporated to dryness using an evaporator, dissolved in 10 ml of distilled water, and bioassayed. In the same manner, 10 g of Diaion HP-20™ (a synthetic adsorbent ion exchange resin with the following chemical structure



) (Mitsubishi Chemical) was suspended in methanol, degassed in a vacuum, and filled into a column. In order to prevent the carrier from floating up, sea sand B was layered over the carrier in the column at a thickness of 5 mm. The eluent in the column was replaced with distilled water, and the column was washed with 100 ml of distilled water. Then, 5.0 ml of CM was put into the column, and eluted with 100 ml of distilled water, 100 ml of 30% CH<sub>3</sub>CN, and 100 ml of 60% CH<sub>3</sub>CN in order (flow rate: 60 ml/hour). The resulting fractions were evaporated to dryness using an evaporator, dissolved in 10 ml of distilled water, and bioassayed.

Please replace the paragraph beginning on page 17, line 10, which starts with “The plant growth”, with the following amended paragraph.

The plant growth factors were not retained by any of Cosmosil 75C<sub>18</sub>-OPN™ and Diaion HP-20™, but were eluted out of the columns with distilled water. It is therefore evident that the present plant growth factors have relatively high polarity.

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Please replace the paragraph beginning on page 18, line 6, which starts with "DEAE", with the following amended paragraph.

DEAE Sephadex A-25™ (0.8 g)(Pharmacia LKB Biotechnology) was swollen in 50 ml of 500 mM Tris-HCl buffer (pH 7.4) at room temperature for 24 hours, then suspended in the same buffer of 20 mM, and filled into a column (1.2 x 3.5 cm; 4.0 ml). Then, 10 ml of CM was lyophilized, and the resulting lyophilisate was dissolved in 10 ml of the same buffer. Then, the solution was put into the column, and eluted with 20 ml of the same buffer, 20 ml of the same buffer containing 250 mM KCl, 20 ml of the same buffer containing 500 mM KCl, and 20 ml of the same buffer containing 1000 mM KCl in order (flow rate: 15 ml/hour). Then, each eluate fraction was concentrated to 10 ml, and injected into a dialysis tube (Spectra/Por 7 MWCO: 1000), the both ends of which were closed with closers. These tubes were put in 3000 ml of distilled water at 4°C for 24 hours for dialysis to desalt the fractions. Each dialysate was concentrated to 10 ml, and then bioassayed.

Please replace the paragraph beginning on page 18, line 18, which starts with "In the same", with the following amended paragraph.

In the same manner, 0.8 g of CM Sephadex C-25™ (a carboxymethyl-moiety containing ion-exchange resin) was swollen in 50 ml of 500 mM KH<sub>2</sub>PO<sub>4</sub>-KOH buffer (pH 6.0) at room temperature for 24 hours, then suspended in the same buffer of 20 mM, and filled into a column (1.2 x 3.5 cm; 4.0 ml). Then, 10 ml of CM was lyophilized, and the resulting lyophilisate was dissolved in 10 ml of the same buffer. The solution was put into the column, and eluted with 20 ml of the same buffer, and 20 ml of the

same buffer containing 250 mM KCl in order (flow rate: 15 ml/hour). Each eluate fraction was desalted through dialysis, and then bioassayed.

Please replace the paragraph beginning on page 19, line 3, which starts with "The present plant", with the following amended paragraph.

The present plant growth factors were very strongly adsorbed to Sephadex A-25™ and eluted with 1000 mM KCl. On the other hand, they were not adsorbed at all to CM Sephadex C-25™. The present plant growth factors were eluted with 20 mM KH<sub>2</sub>PO<sub>4</sub>-KOH buffer. These results suggest that the present plant growth factors are acidic substances.

Please replace the paragraph beginning on page 19, line 10, which starts with "A non-specific", with the following amended paragraph.

A non-specific peptidase, Pronase E™ (3.0 mg)(Sigma), was dissolved in 3.0 ml of 20 mM KH<sub>2</sub>PO<sub>4</sub>-KOH buffer (pH 7.5). The precipitate was removed by filtration through a cellulose acetate filter (ADVANTEC DISMIC-13cp, 0.20 μm) to prepare an enzyme solution. A portion (1.0 ml) of the enzyme solution was heated in a boiling bath for 10 minutes to prepare a deactivated enzyme solution. Into the test tubes were put 0.9 ml of the same buffer, 1.0 ml of CM or distilled water, and 100 μl of the enzyme solution or the deactivated enzyme solution or the same buffer, and shaken in a thermostat shaker (TAITEC, Personal-11) at 37°C at 170 rpm for 3 hours. After the enzymatic reaction, the reaction liquids were adjusted to pH 5.8 with 0.1 N HNO<sub>3</sub>, then heated in a boiling bath for 10 minutes, and thereafter immediately cooled with ice,

thereby deactivating the enzyme. After the deactivation, these samples were bioassayed.

Please replace the paragraph beginning on page 20, line 13, which starts with "The present plant growth", with the following amended paragraph.

The present plant growth factor were completely deactivated when treated with the peptidase Pronase E™. Accordingly, it is evident that these factors have a peptide structure in their molecule, and this peptide structure moiety is important for the factors to express their activity.

Please replace the paragraph beginning on page 21, line 7, which starts with "CM is a polar", with the following amended paragraph.

CM is a polar substance, and is not retained in reversed-phase columns with Cosmosil 75C<sub>18</sub>-OPN™ and Diaion HP-20™.

Please replace the paragraph beginning on page 21, line 12, which starts with "CM is deactivated", with the following amended paragraph.

CM is deactivated by Pronase E™, but its activity is still kept even when treated with Glycosidases "Mixed".

Please replace the paragraph beginning on page 21, line 15, which starts with "CM is strongly", with the following amended paragraph.

CM is strongly adsorbed to DEAE Sephadex A-25™ (and eluted with 1000 mM KCl), but it is not adsorbed at all to CM Sephadex C-25™.

Please replace the paragraph beginning on page 21, line 19, which starts with "DEAE Sephadex", with the following amended paragraph.

DEAE Sephadex A-25™ (3.0 g) was swollen in 50 ml of 500 mM Tris-HCl buffer (pH 7.4) at room temperature for 24 hours, suspended in the same buffer of 20 mM, and filled into a column (1.7 x 8.0 cm; 18 ml). Then, 100 ml of CM was concentrated to 50 ml using an evaporator, and Tris was added thereto at a final concentration of 20 mM. The solution was adjusted to pH 7.4 with 6 N HCl. Then, CM was put into the column, and six fractions were eluted as follows: with 30 ml of the same buffer, 30 ml of the same buffer containing 250 mM KCl, 30 ml of the same buffer containing 500 mM KCl, 30 ml of the same buffer containing 750 mM KCl, 30 ml of the same buffer containing 1000 mM KCl, 30 ml of the same buffer containing 1250 mM KCl, and 30 ml of the same buffer containing 1500 mM KCl. The active fractions were estimated from their UV absorbance at 220 nm.

Please replace the paragraph beginning on page 22, line 15, which starts with "The desalted", with the following amended paragraph.

The desalted eluates obtained from the DEAE Sephadex™ column (fractions eluted with 1000 mM KCl and 1250 mM KCl) were lyophilized, and dissolved in 1.0 ml of 20 mM KH<sub>2</sub>PO<sub>4</sub>-KOH buffer (pH 5.8). Then, the resulting solution was put into a Bio-Gel P-2™ extra fine column (1.7 x 37 cm) which was first equilibrated with the same buffer as that used for the previous dissolution. While monitoring the UV absorbance at 220 nm, the same buffer was applied to the column at a flow rate of 15 ml/hour (Fig. 3-b).

Please replace the paragraph beginning on page 23, line 4, which starts with "The active eluate fraction", with the following amended paragraph.

The active eluate fraction obtained from the Bio-Gel™ column was lyophilized, and then dissolved in 10  $\mu$ l of 10% acetonitrile containing 0.1% TFA. The resulting solution was put into a Develosil ODS-HG-5™ column (4.6 x 250 mm, produced by Nomura Chemical), and chromatographed according to isocratic elution with 10% acetonitrile containing 0.1% TFA at a flow rate of 1.0 ml/min, while monitoring the UV absorbance at 220 nm. Fractions of 2 ml each were collected, and their activity was determined through bioassay (Fig. 3-c). Two active fractions were collected. The yields from 600 ml of CM were 2  $\mu$ g of compound (I-1) and 10  $\mu$ g of compound (I-2). 10<sup>7</sup>-fold purification from CM was attained, and the recovery of the activity was 10%.

Please replace the paragraph beginning on page 24, line 7, which starts with "The observation", with the following amended paragraph.

The observation of the fragment ions (M - H + 80)<sup>-</sup> in the FAB-MS experiments indicated that compound (I-1) was a sulfated compound (Rogers et al.; Carbohydr. Res. Vol. 179, pp. 7-19, 1988), and the additional 160 mass units of compound (I-1), by which the molecular weight of compound (I-1) as measured through FAB-MS analysis was larger than the estimated molecular weight of the compound as calculated on the basis of the amino acid structure, were suggested to be sulfate groups with which the OH groups of the two tyrosine residues were substituted. Since the sulfate-substituted tyrosines are adsorbed to DEAE Sephadex™, and the sulfuric acid moieties are



removed under the condition for amino acid sequencing, the structure of compound (I-1)  
was determined.

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